Title: Endocrine, sexual and reproductive functions in patients with Klinefelter Syndrome compared to non-obstructive azoospermic patients.

Abstract

Aims: We aimed to investigate fertilization rates, quality of embryo, pregnancy and live birth rates, endocrine, sexual function, psychological status and quality of life of cases diagnosed with Klinefelter syndrome (KS).

Methods: Clinical findings, hormone values and semen analyses in patients with nonmosaic KS (Group 1, n=121) and those with non-genetic nonobstructive azoospermia (NOA) (Group 2, n=178) were retrospectively analyzed. Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE), fertilization rates and embryo quality, pregnancy, abortion, and live birth rates were compared. Sexual functions were assessed using IIEF-15, quality of life was evaluated, and psychological status was assessed.

Results: There was no difference in terms of age between groups. Sperm retrieval rates was 38% and 55.6% in Group 1 and 2, respectively (p=0.012). Sperm retrieval rates were higher in Group 1 before 31.5 years than in Group 2 (AUC=0.620, 0.578). Compared to Group 2, the fertilization rate was low in Group 1, whereas embryo quality was similar. Live birth rates were 12.5% and 23% in Group 1 and 2, respectively (p=0.392). The education level, libido, erectile functions, and general health satisfaction were lower in Group 1 than in Group 2 (buraya p değeri yaz). Depression and anxiety levels were higher in Group 2 than Group 1 (p değeri yaz).

Conclusion: Higher sperm retrieval rate has been achieved in group 1 younger than 31.5 years. Similar embryo quality is provided between groups. Sexual dysfunction and psychiatric problems were higher in Group 1, with lower satisfaction and general health than Group 2. Patients with KS should be monitored not only with their reproductive functions but also with their general health status.

Key Words: Fertility, Klinefelter Syndrome, psychology, quality of life, sexual function, non-obstructive azoospermia.

What is already known about this topic?

1. Klinefelter Syndrome (KS) is the most common chromosomal disorder in men.

2.Men with KS generally have infertility.

2. Newborns with KS are similar to healthy babies phenotypically, and adolescents see tall height, long legs relative to the body, atrophic-small testicles, feminine body structure, and gynecomastia.

What does this article add?

1. This study compares to a high number of patients with Klinefelter Syndrome in terms of endocrine parameters, sexual and reproductive functions, and life quality.

2. When the age is set to 31.5 years, a higher sperm retrieval rate could be reached.

3. Due to the low libido, erectile dysfunction, anxiety, depression and dissatisfaction with general health conditions, KS patients should be considered for lifelong endocrinological monitoring in addition to testosterone replacement treatment.

Introduction

Klinefelter syndrome (KS) is the most common sex chromosomal disorder in men phenotypically. It is characterized by tall height, long legs relative to the body, atrophic-small testicles, feminine body structure and gynecomastia. Its prevalence is 1/650 [1]. Newborns with KS are similar to healthy babies [2]. Classical testicular atrophy occurs with puberty [3]. Laboratory and clinical findings in adulthood are consistent with hypergonadotropic hypogonadism. High serum FSH level is the leading laboratory finding. The definitive diagnosis is made by karyotype analysis. Of the KS patients, 90% have nonmosaic 47, XXY, 10% 46, XY / 47, XXY mosaic chromosome establishment and other numerical and structural anomalies such as 47, iXq, Y karyotype [4]. The X chromosome contains more than 1100 genes that play a role in many systems, including testicular function, brain development and growth [5]. Additional X chromosome inactivation is initiated at the X chromosome inactivation center (XIC) by activating the XIST promoter. Since many genes on the X chromosome are highly expressed in the testicles, ovaries and brain, these organs are affected by the X chromosome polysomy [6]. Of the patients with KS, 11% azoospermic, and 4% undergo infertility investigation [7], 10% of the KS patients are prenatally diagnosed, 3% in childhood due to developmental delay and behavioural problems, 2% in puberty due to delayed puberty and gynecomastia, and 17% in adulthood hypogonadism and infertility [1]. Testicular histology is characterized by decreased or complete apoptosis of the germ cells. Hyalinization and atrophy in the seminiferous tubules, fibrosis in the interstitium are frequently observed, but spermatogenesis can be observed in small areas [8]. A definitive

treatment to correct spermatogenesis has not been defined yet. The typical finding in semen analysis is azoospermia. In azoospermic nonmosaic individuals, fertilization can be achieved with micro-TESE and intracytoplasmic sperm injection (ICSI). However, severe oligozoospermic patients can have children with IVF, TESE, micro-TESE and ICSI (7). Social skills disorder, language development, communication, adaptation, retardation of attention [9], anxiety, depressive disorder [1], schizophrenia and bipolar disorder [10] and learning difficulties is higher in KS patients [11]. Libido decreases, and the incidence of premature ejaculation (PE) is low [12].

This study aimed to compare the endocrine parameters, sexual and reproductive functions, and the quality of life in KS patients compared to nonobstructive azoospermic (NOA) patients.

Materials and Methods

After obtaining ethical approval of Ondokuz Mayıs University, Clinical Research Ethics Committee (18.01.2019/B.30.2.ODM.0.20.08 / 33), between 2012 and 2019, this retrospective study was carried out in the Andrology clinic and IVF center of Ondokuzmayıs University. Of the patients, 121 with nonmosaic Klinefelter syndrome were assigned to Group 1 and 178 with nonobstructive azoospermia (NOA) were assigned to Group 2.

Patients' data were obtained from the medical records. Informed consent was obtained from all cases. The diagnosis of KS was made by karyotype analysis using the high-resolution method with methotrexate and thymidine (MTX) synchronization from peripheral blood.

Demographic data, medical history and physical examination findings were recorded. Testicular volume was measured by Prader orchidometry (Plastic OM20, Erler-Zimmer, Germany). Semen analysis was performed at least twice with an interval of at least a month. The diagnosis of azoospermia was made by the World Health Organization (WHO) 2010 guideline definition, with the absence of sperm in the examination of the pellet obtained after centrifugation at 3000 g for 15 minutes at X400 magnification [13].

The diagnosis of NOA was made with the presence of bilateral palpable vas and epididymis, high serum gonadotropin levels and normal ejaculate volume, and azoospermia in semen analysis [14].

Blood samples were taken between 07-11 A.M for hormonal analysis. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), Estradiol (E2) and total testosterone (TT) levels were measured by radioimmunoassay (RIA) method. Karyotype and Y chromosome microdeletion analyzes were performed using the MTX synchronization method with lymphocyte cultures isolated from heparinized peripheral blood. The analysis

was performed at 20 metaphases using G-banding and 550-700 band resolution. Mosaicism was evaluated by FISH method, and Y chromosome microdeletion analysis was performed by multiplex PCR method by obtaining DNA from peripheral blood samples [15].

The results were reported according to the international human cytogenetic naming system (ISCN) [16]. Sperm was searched by the micro-TESE method in Groups 1 and 2. Sperm retrieval and fertilization ratios, embryo qualities, transferred embryo data, pregnancy, abortion and live birth ratios were recorded.

Surgical technique

Under local anesthesia, the scrotum and its layers were opened with a midline vertical raphe incision, the tunica vaginalis was opened, the testis was born out of the incision, and the testicular parenchyma was explored by opening the tunica albuginea up to the hilum of the testis. The equatorial incision was performed, tracing the avascular area. Antihilar longitudinal incision was applied to testes smaller than 6 mL. Testicular parenchyma was dissected at X24 magnification under the biomicroscope (ZEISS OPMI S5, Germany), and primarily subtunical and perivascular areas were scanned. Dilated and opaque tubules were isolated and excised using micro-forceps (Fig. 1). The surgery period was recorded. The tunical incision was closed with a 5/0 polyglactin running suture (Vicryl®, Ethicon, USA). The testis was placed in the scrotum, and the scrotum and its layers were closed. The excised seminiferous tubule samples were placed in a petri dish containing MOPS buffer (G-mops plus, Vitrolife®, Switzerland) and sent to the embryology laboratory. The specimen was mechanically dissected with an insulin injector needle under a stereomicroscope (OLYMPUS SZX7, Japan). Sperm was searched under an inverted microscope (ZEISS AXIO Observer. A1, Germany) at X40 magnification (Fig. 2). Once spermatozoa were found, the sample was prepared for intracytoplasmic sperm injection. The pellets were centrifuged at 600 g for 10 minutes after an enzymatic process (Collagenase, Type 1A, Sigma, USA) for about 90 minutes at 37 ° C. When samples contained no spermatozoa, the examination was repeated for the other testicle.

Sperm processing for ICSI

The liquid portion of the suspension containing sperm in a sterile tube was subjected to dense gradient centrifugation at 600 g for 10 minutes. High and low phase gradients (SpermGrad-30®, Vitrolife, Switzerland) and the liquid portion were sequentially placed in a sterile conical tube with a transfer pipette and centrifuged. The supernatant was discarded, and the pellet was resuspended with sperm washing liquid (SpermRinse®, Vitrolife, Switzerland) and centrifuged at 300g for 10 minutes. After the supernatant was removed, the remaining pellet

was resuspended in 0.5 ml sperm washing liquid, and one drop of it was examined under a microscope for spermatozoa.

ICSI

An inverted microscope with Narishige Micromanipulator attachment (ZEISS AXIO Observer. A1, Germany) was used. Prepared Petri plate with MOPS (G-mops plus, Vitrolife®, Switzerland) solution was used, where the collected oocytes and sperms will be placed. Spermatozoa with normal morphology were taken from the sperm-drop using an ICSI pipette (Vitrolife®, Switzerland) and transferred to the medium containing polyvinyl pyrrolidone (PVP, ICSI®, Vitrolife, Switzerland). After the oocyte was fixed with a holding pipette (Vitrolife, Switzerland), the selected sperm was transferred to the oocyte cytoplasm with the help of an ICSI pipette. After the ICSI procedure, artificial oocyte activation was performed with calcium ionophore (Calcium ionophore, A23187, Sigma, USA) to increase the fertilization rate [17]. After ICSI, oocytes were transferred to a culture medium and incubated (Sanyo, MCO-18M, Japan). Embryo development was monitored until the day of transfer.

Embryo Selection and Embryo Transfer

According to the number and developmental potential, embryos in cleavage or blastocyst periods were selected, and the transfer procedure was performed. Cleavage scoring (Table 1) [18] on the 3rd day and Gardner's blastocyst scoring systems [19] on the 5th day were used for the selection of the embryo to be transferred (Figure 3). The main criteria for scoring were the number of blastomeres, symmetricity and fragmentation for the Cleavage period. For the blastocyst stage, expansion, inner cell mass and cell amounts in the trophectoderm layer were accepted as scoring criteria [19].

Pregnancy Follow-up After Embryo Transfer

Measurement of serum beta-hCG level being at least five mIU / ml on the 12th day of embryo transfer was accepted as positive pregnancy, non-visualized gestational sac despite the high serum beta-hCG was accepted as biochemical pregnancy and presence of fetal elements, and heart movement in transvaginal ultrasonography was accepted as clinical pregnancy. The presence of a fetus less than 500 g and pregnancy that ended within the first 20 weeks was considered abortion. Ectopic pregnancy was diagnosed ultrasonographically in the presence of a gestational sac located outside the uterine cavity. Babies born alive at 24 weeks and over 500 grams were accepted as live births.

Sexual Function, Quality of Life, Psychological Status

Erectile function, orgasmic function, libido, sexual satisfaction and general satisfaction of the patients were assessed using the IIEF-15 questionnaire form consisting of 15 questions [20]. Quality of life was assessed using the World Health Organization Quality of Life Score short form (WHOQOL- Bref) [21], and psychological condition was investigated prospectively using the Beck Anxiety and Depression Scale [22], by phone or by e-mail. Erectile dysfunction was classified as severe ED (0-10 points), moderate ED (11-16 points), mild-moderate ED (17-21 points), mild ED (22-25 points). Patients with IIEF score >26 were considered non-ED. Physical, mental, social and environmental well-being was evaluated with the WHOQOL-Bref scale consisting of 26 questions. The first two questions assess the general quality of life and health status. This scale, which has five options for each question evaluating each field independently, was prepared as 1: not satisfied, 5: very satisfied, and the field scores were calculated between 4-20. The higher the score, the higher the quality of life. Beck Anxiety Scale consists of 21 symptom categories; each has four Likert-type options scored between 0 and 3. The high score obtained indicates the severity of the anxiety (Total score = 0–7 points=normal, 8–15 points=minimal, 16–25 points=moderate, 26–63 points=severe anxiety). In this study, a cut-off level of 17 was accepted for depression [23].

Statistical analysis

Statistical analysis was performed using IBM Statistics SPSS 22 (2012, Chicago, USA) package program. Kolmogorov Smirnov test was used for normally distributed data. Mann-Whitney U test was used to assess the differences between the groups. Chi-square test was used for comparing categorical data according to groups. ROC analysis was used to determine the cut-off point. Analysis results were presented as median, minimum, maximum for quantitative data and as frequency and percentage for categorical data. A p-value of <0.05 was considered statistically significant.

Results

The demographic and clinical findings are shown in Table 2. Testicular volume and total testosterone level were low, but FSH and LH levels were high in Group 1 than Group 2 ($p <0.001$). A higher sperm retrieval rate was reached in Group 1 than Group 1 ($p=0.0012$) (Table 2). According to age groups, the sperm retrieval ratio was high in Group 2 ($p = 0.023$). Sperm retrieval rate increased in patients with KS between 26 and 35 (p=değeri Yaz) (Table 3). When the cut-off point was considered 31.5 years, the ROC analysis showed a higher sperm retrieval rate in Group 1 than Group 2 (Figure 4). Data on sensitivity, specificity, positive and negative predictive values are shown in Table 2. In Group 2, the greater testicular volume was associated with an increased rate of sperm retrieval ($p = 0.017$).

Only the second question was statistically different (p=0.006) (bu ne demek) in Group 2, whereas the remainings were similar between groups (p> 0.05). The education level was higher in Group 2 than Group 1 (p = 0.001). The mean erectile function and libido scores were better in Group 2. The mean anxiety score was high in Group 2 (p = 0.001). The mean depressive symptom scores were 51.1% and 5% in Group 1and 2, respectively (Table 2).

As testicular volume increased in group 1, sperm retrieval rate increased (p = 0.017). FSH, LH, TT levels, and aromatase inhibitor's use did not affect sperm retrieval rates in both groups (p> 0.05).

The data of one of the three patients in Group 1 were not available, and no sperm was found after centrifugation in two cases. Fertilization and cleavage rates of patients according to different age groups were lower in Group 1 than Group 2 (p> 0.05) (Table 4). Thirty-eight embryos in Group 1 and 177 embryos in Group 2 were evaluated using the Cleavage Scoring System. Embryo transfer was performed on the third day in 19 and 82 patients in Group 1 and 2. Embryos were transferred on the fifth day in two and nine patients in Group 1 and 2. Embryo qualities were similar between groups (p = 0.816) (Table 5).

Fertility data on embryo transfer are given in Table 6. Clinical pregnancy was detected in three (12.5%) of 24 patients with KS and 23 (25.3%) of 91 cases with NOA. Fertility, pregnancy and live birth rates were similar (p> 0.05) (Table 6).

Discussion

The majority of KS patients are azoospermic and were considered infertile until recently. Additional X chromosome initiates early testicular development that accelerates germ cell loss, and the fibrotic process occurs during puberty. However, focal spermatogenesis in seminiferous tubules has been reported in 20-69% [24]. The present study showed 38% and 55.6% sperm retrieval rates in Group 1 and 2. The low number of sperm retrieval rates in Group 1 can be attributed to the disease's nonmosaic form. Previous reports stated the sperm retrieval rates were 54.5% and 16% in patients with mosaic and nonmosaic forms, respectively [25]. Age, testicular volume and hormonal status of the patients, preoperative medical treatment, surgical technique and surgeon's experience may play a role in increasing sperm retrieval rate in patients with KS.

The effect of age on sperm retrieval rate in patients with KS is controversial. Some researchers suggested that the success rate for sperm retrieval decreases with age [26], and the critical range was 32-35 years [27]. In contrast, others advocated that the sperm retrieval rate was low in early puberty [28]. However, most studies have shown that age did not affect the sperm retrieval rate [29], as shown in the present study. As the age increases, the number of

spermatogonial cells in testicles decreases and DNA damage increases [30]. In KS, a diffuse fibrotic process that starts with puberty in the testis affects the testicles as the age progresses [29]. Therefore, the possibility of sperm presence decreases in advanced ages. Although a direct relationship has been shown between testicular volume and spermatogenesis in normal individuals [31], controversy still exists for KS patients [27]. The mean testicular volume of nonmosaic adult CS patients was reported less than 4 ml [32], as shown in the present study. Our results showed that KS patients had larger testicular volumes than patients with NOA.

Serum FSH and LH levels in patients with KS increase starting from the middle of puberty [33], serum testosterone level remains below average in most cases around the age of 25 [1]. In this study, the hormonal results of patients with KS were compatible with the literature. Besides studies showing that hormonal status did not affect micro-TESE success in KS patients [34], some other studies showed that serum testosterone above 7.5 nmol / L and LH level below 17.5 U / l increases sperm retrieval rate. There are also studies [26]. Our results showed no relationship between hormone levels and sperm retrieval rates.

Some authors investigated the effect of aromatase inhibitors on sperm retrieval success on the ground that serum testosterone levels above 300 ng / dL and high intra-testicular testosterone levels would increase the likelihood of sperm retrieval [35]. Aromatase inhibitors increase the serum testosterone level and the testosterone/estradiol ratio by inhibiting peripheral testosterone conversion to estradiol. Some researchers suggested that pre-TESE use of aromatase inhibitors increased sperm retrieval success [36], while others did not [37]. In our study, aromatase inhibitor treatment given at least three months before micro-TESE had no impact on sperm retrieval success. Sertoli cells regulate spermatogenesis via testosterone and FSH [38]. Intratesticular testosterone is one of the most critical factors mandatory for spermatogenesis initiation and maintenance [39]. It has been suggested that intratesticular testosterone levels are higher in KS patients than in the normal population. Low peripheral serum testosterone is caused by the inadequate release of testosterone into the systemic circulation due to the decrease in vascular/testicular surface and testicular fibrosis [40].

In assisted reproductive technologies, sperm and oocyte quality affects the fertilization rate. Similar fertilization rates (48.0% - 52.7%) have been given for patients with KS, NOA and normal karyotype [41].

In our study, no relationship was found between age and fertilization and cleavage rates in patients with KS and NOA. Also, the rate of progression of the fertilized oocyte to the cleavage period was significantly lower in Group 1 (80%) compared to Group 2 (100%). This may be due to the different oocyte quality (female factor). In English literature, only a limited

number of studies investigated the quality of embryos obtained from KS patients. In two different studies, fertilization rate and cleavage rates of embryos obtained from fresh and frozen sperm were similar in patients with KS [42]. Our results on all embryos' quality on the 3rd day after ICSI obtained from fresh sperm in Groups 1 and 2 were similar.

Sexual problems such as ED secondary to hypogonadism and decreased libido can be seen in patients with KS. In a study, the erectile dysfunction (ED) rate (18.9%) was similar among the patients with KS and the age-matched control group. In a study, normal erectile function was reported in KS patients with a decreased libido and a low incidence of premature ejaculation [12].

Our results on sexual functions showed that KS patients had a higher ED rate and decreased libido than NOA patients. This may be attributed to a lower testosterone level in the KS group compared to the NOA group.

It has been reported that language development, communication, adaptation, and attention problems are more common in cases with KS and social skills disorder [44]. Besides, psychiatric disorders, such as anxiety and depression, are more common [45].

Although some authors reported that academic performance and professional status were lower than individuals with similar socio-economic status [11], some advocated that most individuals with KS had an average range of intellectual abilities, behaviour, attention, social skills and functionality. Increased risk of anxiety and depression were found in KS patients compared to NOA patients.

To date, only limited studies have investigated the quality of life of KS patients. Lower mental and quality of life scores were reported in KS patients compared to controls [45]. The present study showed that Group 1 patients were less satisfied with their health status than Group 2, but the quality of life remained similar.

Strengths & Limitations

This study's main strength is investigation of a high number of patients with Klinefelter Syndrome in terms of endocrine parameters, sexual and reproductive functions, and the quality of life.

Limiting factors are;

1. Lack of endocrine and fertility data in some cases is one of the limiting factors,
2. Retrospective nature of the study covering only patients with KS living in the Central Black Sea Region,
3. The inability to access complete data of the patients who continue their treatment at an external center,

4. Small number of cases, and the inability to evaluate the female factor and laboratory conditions,

5. Participation of a small number of patients in the study to evaluate the psychological state, sexual function and quality of life.

Conclusions

KS, which is an important cause of male infertility, is a clinical entity that should be considered primarily in the differential diagnosis of NOA patients. When the critical age cut-off is set to 31.5, 38.0% of sperm retrieval rate was reached using the micro-TESE in patients with KS, and 12.5% of the couples had a live child. This study showed that the embryo quality and live birth rate were similar between KS and NOA patients; the rate of sperm retrieval increased as the testicular volume increased in the KS arm. The hormonal status and aromatase inhibitor treatment did not increase the sperm retrieval rate. Due to the low libido, ED, anxiety, depression and dissatisfaction with general health conditions in KS patients, lifelong endocrinological monitoring should be taken, and testosterone replacement treatment should be given when necessary.

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Figure legends

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Fig. 2. Spermatozoa view under inverted microscope (black arrow, X40 magnification)

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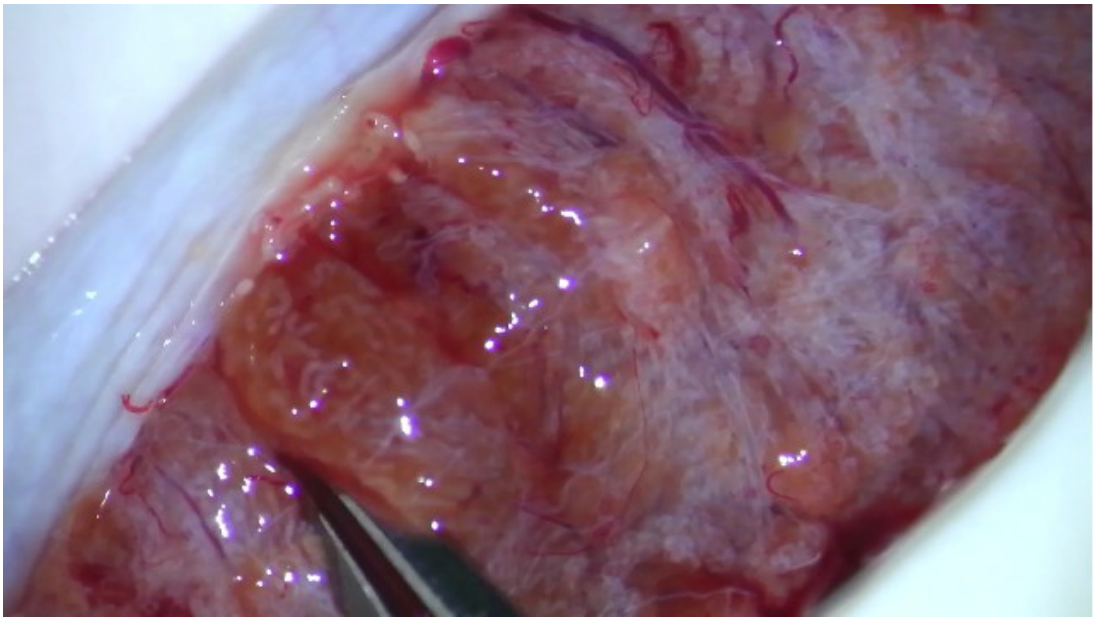

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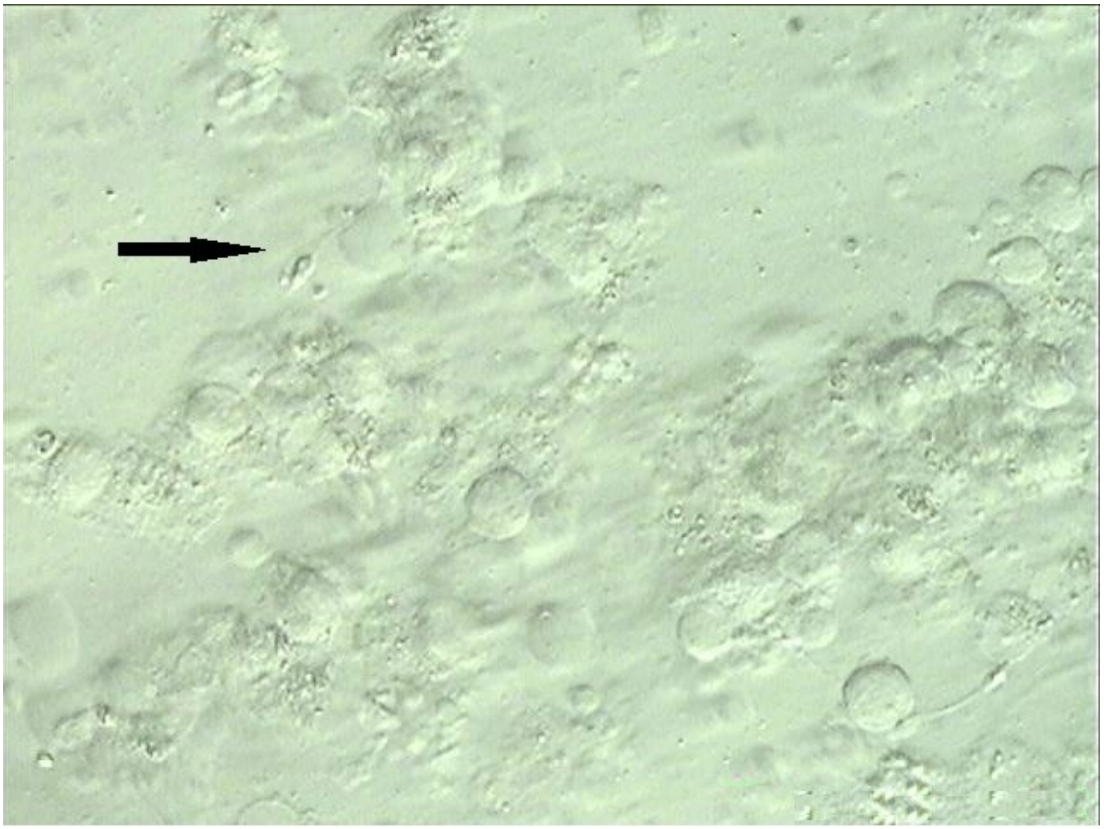

Fig. 2. Spermatozoa view under inverted microscope (black arrow, X40 magnification)

Table 1. Cleavage Scoring System

Embrio Grade	Definition
Grade 1	Equal-sized blastomeres, no fragmentation
Grade 2	Equal-sized blastomeres, little fragmentations
Grade 3	Blastomeres are unequal; no or very little fragmentations
Grade 4	Blastomeres equal or not, intense fragmentation
Grade 5	Different sizes of blastomeres, intense or complete fragmentation

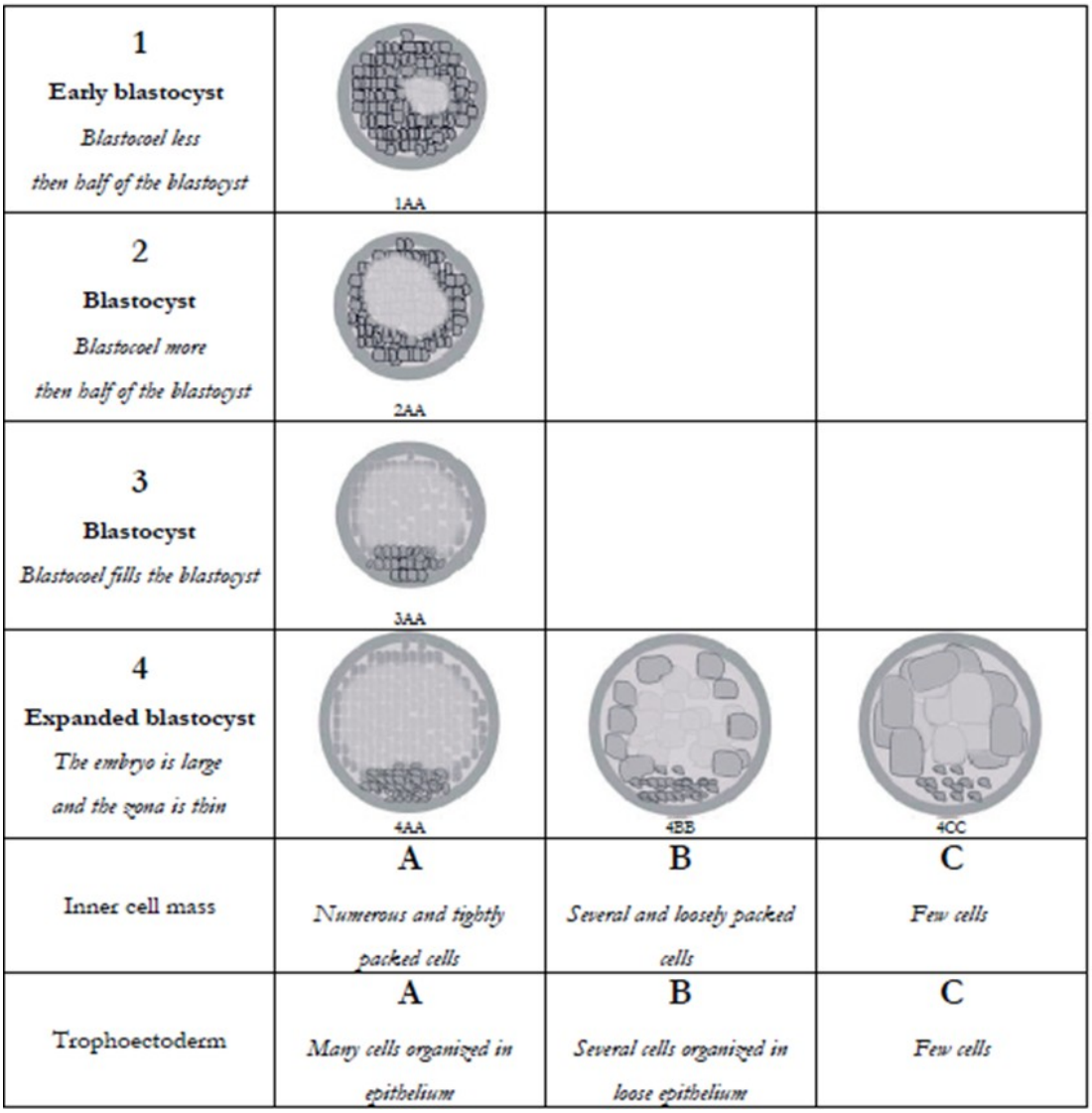

Fig. 3. Gardner's Blastocyst Scoring System

Table 2. Demographic, clinical and surgical results of the groups.

	KS* Group (n=71)	NOA** Group (n=178)	p

Age (median, min-max)	32.0 (23-44)	32.0 (21-57)	0.393
Testicular volume (mL) (median, min-max)	4.0 (1.0-15.0)	14.0 (4.0-32.0)	0.001
Hormones (median, min-max)			
FSH (mU/mL) ***	38.0 (6.0-86.9)	17.9 (2-63.4)	0.001
LH (mU/mL)#	22.5 (4.3-43.5)	8.9 (1.6-32.3)	0.001
Estradiol (pg/mL)	26.1 (5.0-69.8)	11.8 (3.5-72.9)	0.169
Prolaktin (ng/mL)	11.5 (2.8-52.3)	23.0 (5.0-71.7)	0.720
Total testosteron (ng/mL)	2.4 (0.2-15.5)	3.6 (0.3-12.3)	0.001
Surgery time (min)	104 (100-110)	86 (80-95)	0.001
Spermatozoa presence in micro-TESE (n, %)			
Yes	27 (38.0)	99 (55.6)	0.012
No	44 (62)	79 (44.4)	0.012
Results of ROC analyses (cut-off=31.5 years)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	-
Area under curve	59.3	57.8	
Sensitivity	56.8	52.0	
Specificity	45.7	53.9	
Positive predictive value	69.4	52.9	
Negative predictive value	57.8	53.4	
Odds ratio			0.400
WHOQOL+-Bref first question (n,%)			
Vary bad	3 (6.7)	17 (13.6)	
Bad	2 (4.4)	7 (5.6)	
50% good	23 (51.1)	47 (37.6)	
Rather good	14 (31.1)	43 (34.4)	0.006
Very good	3 (6.7)	11 (8.8)	
WHOQOL-Bref second question (n, %)			
Not satisfied	3 (6.7)	8 (6.4)	
Little satisfied	9 (20.0)	8 (6.4)	
50% satisfied	17 (37.8)	17 (13.6)	
Rather satisfied	11 (24.4)	69 (55.2)	
Very satisfied	5 (11.1)	23 (18.4)	
WHOQOL – Bref subdomains (n, min-max))			0.237
Physical	28 (17-35)	26 (18-34)	0.131
Psychogenic	22 (9-30)	23 (14-29)	0.084
Sosyal	11 (3-15)	11 (7-15)	0.894
Çevre	27 (15-40)	27 (22-37)	
Psychogenic status			0.001
Beck Anksiyete Ölçeği (n, %)			
Minimal	13 (28.9)	111 (88.8)	
Mild	12 (26.7)	8 (6.4)	

Moderate	9 (20.0)	3 (2.4)	
Severe	11 (24.4)	3 (2.4)	
Beck Depresyon Inventory (n, %)			0.001
Depressve symptoms are present	23 (51.1)	13 (10.4)	
No depressive symptoms	22 (48.9)	112 (89.6)	
Educational status (n, %)			0.001
Preliminary	25 (55.6)	29 (23.2)	
High school	15 (33.3)	31 (24.8)	
University	5 (11.1)	65 (52.0)	
Sexual functions			
Erectile dysfunction (n, %)			0.012
No	18 (40)	111 (88.8)	
Mild	10 (22.2)	8 (6.4)	
Mild to moderate	5 (11.1)	3 (2.4)	
Moderate	6 (13.3)	3 (2.4)	
Severe	6 (13.3)	-	
Libido (IIEF-short form, 11. and 12. questions)	7.10	8.49	0.010

[*]KS, Klinefelter Syndrome; [**]NOA, non-obstructive azoospermia; [***]FSH, follicle-stimulating hormone, [#]LH, luteinizing hormone; [+]WHOQOL, World Health Organization Quality of Life questionnaire.

Table 3. Presence of spermatozoa according to different age ranges in groups.

	KS Group			NOA Group		p
Age ranges	Yes (n=27)	No (n=44)	p	Yes (n=99)	No (n=79)	
18-25	3 (11.1%)	3 (6.8%)		3 (3.0%)	7 (8.9%)	
26-30	10 (37.0%)	13 (29.5%)	0.083	25 (25.3)	32 (40.5)	0.023
31-35	12 (44.4%)	13 (29.5%)		41 (41.4)	20 (25.3%)	
36 and older	2 (7.4%)	15 (34.1%)		30 (30.3)	20 (25.3%)	

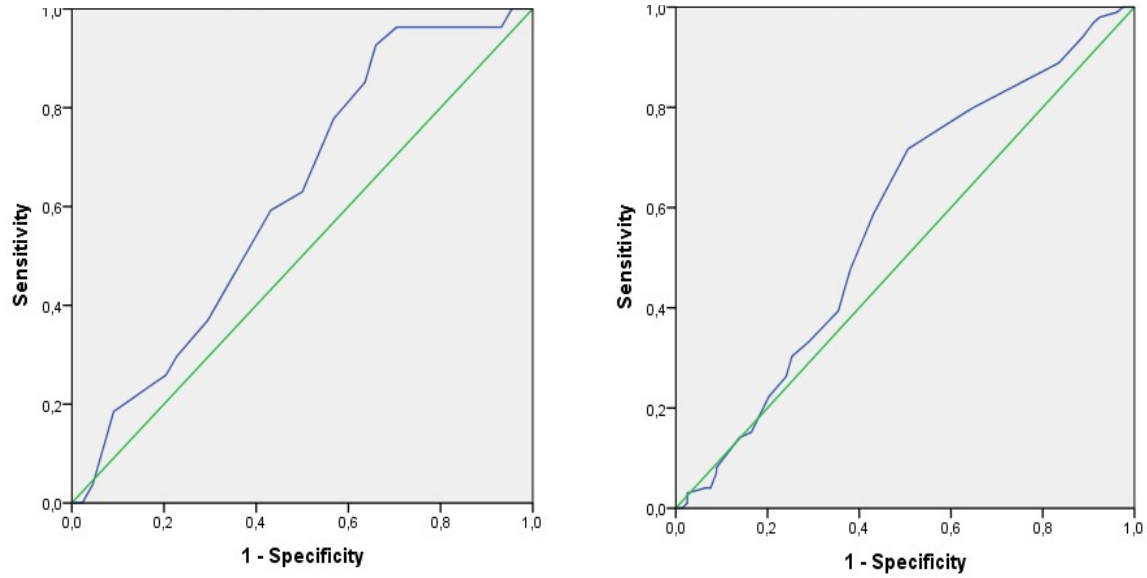

Fig. 4: ROC analyses of patients with KS and NOA.

Table 4. Intracytoplasmic sperm injection outcomes of the patients with KS and NOA.

	KS (n=24)	NOA (n=91)	p
Obtaine oocyte (median, min-max)	13.0 (6-28)	10 (2-34)	0.075
Mature oocyte (median, min-max)	7.0 (2-27)	7 (0-28)	0.314
Number of fertilization (median, min-max)	4.0 (0-17)	4.5 (0-18)	0.511
Fertilization rate (%)	0.5 (0-1)	0.7 (0-2)	0.020
Cleavage number (median, min-max)	5.0 (0-13)	4 (0-17)	0.861
Cleavage rate (%)	0.8 (0-1)	1 (0-1)	0.007
Transfer number (median, min-max)	2.0 (0-2)	2 (0-2)	0.642
Transfer 1 (median, min-max)	1.0 (0-3)	1 (0-4)	0.878
Transfer 2 (median, min-max)	2.0 (1-4)	2 (1-4)	0.824

Tablo 5. Comparison of quality of embrios at third day in KS and NOA.

	KS (n=38)	NOA(n=177)	p
Grade 1	11 (28.9)	51 (28.8)	0.816
Grade 2	14 (36.8)	59 (33.3)	
Grade 3	11 (28.9)	49 (27.7)	
Grade 4	2 (5.3)	18 (10.2)	

Table 6. Outcomes of the patients after embryo

transfer.

	KS (n=24)	NOA (n=91)	p
Biochemical pregnancy	0	8 (8.8)	0.352
Clinical pregrancy	3 (12.5)	26 (28.6)	0.446
Ectopic pregnancy	0	1 (1.1)	-
Abortus	0	2 (2.2)	-
Live birth	3 (12.5)	23 (25.3)	0.392